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IL-13 as Well as IL-4 Induces Monocytes/Macrophages and a Monoblastic Cell Line (UG3) to Differentiate into Multinucleated Giant Cells in the Presence of M-CSF

Takashi Ikeda,* Kazuma Ikeda,† Kazunori Sasaki,* Kimihiro Kawakami,* Kiyohiko Hatake,‡ Yoshio Kaji,§ Hiromichi Norimatsu,§ Mine Harada,† and Jiro Takahara*

*First Department of Internal Medicine, §Department of Orthopedic Surgery, Kagawa Medical University, Kagawa, Japan; †Division of Blood Transfusion, Okayama University Medical School, Okayama, Japan; and ‡Department of Internal Medicine, Jichi Medical School, Tochigi, Japan

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The formation of multinucleated giant cells (MGCs) from monocytes/macrophages is controlled by various cytokines whose crucial roles are not fully understood. In this study, we found that interleukin (IL)-13 as well as IL-4 induced peripheral blood monocytes (PBMs) and monoblastic cell line, UG3, to differentiate into MGCs in the presence of macrophage colony-stimulating factor (M-CSF), while IL-2, IL-7 or IL-10 did not. The presence of M-CSF was essential to this MGC formation, because IL-3 or granulocyte-macrophage colony-stimulating factor (GM-CSF) could not replace M-CSF. IL-4 and IL-13 have been known to inhibit the formation of osteoclast-like cells in the presence of stroma cells or osteoblastic cells. But in our system without stroma cells, IL-4 or IL-13 induced some of characteristics of osteoclasts such as tartrate-resistant acid phosphatase (TRAP) activity, vitronectin receptor (vit-R) expression and resorptive activity for hydroxyapatite, but not the expression of receptors for parathyroid hormone or calcitonin. These results suggest possible involvement of IL-4 and IL-13 in MGCs and osteoclasts development, and UG3 may be useful to further investigate the roles of IL-4 and IL-13 in the formation and physiology of MGCs, and the relationship between these MGCs and osteoclasts.

IL-4 and IL-13 are secreted from Th2 lymphocytes (1), and have significant homology in protein sequence (2). IL-4 and IL-13 have anti-inflammatory activities for monocytes/macrophages such as suppression of the secretion of proinflammatory cytokines, growth factors and chemokines (1-3). IL-4 plays an important role in formation of MGCs from monocytes/macrophages in vivo (4) and in vitro (5-9). IL-13 also induces human monocyte clustering, and it has been hypothesized that IL-13 induces formation of MGCs (10). Recently, it was reported that IL-13 induces monocytes/macrophages to fuse independent of other growth factors (6).

In vivo, monocyte/macrophage-derived MGCs are observed as osteoclasts (11) or so-called foreign body type MGCs (FBMGCs) (12). Osteoclasts are the primary bone resorbing cells, expressing TRAP, a high level of carbonic anhydrase II, calcitonin receptor (cal-R) and vit-R (13). On the other hand, FBMGCs, recognized as macrophage polykaryons, are observed under conditions of hyperimmunity such as infection, rheumatoid arthritis, neoplasia and foreign body reaction against implants (14). FBMGCs may represent down regulation of chronic active inflammation, or participate in frustrated phagocytosis of foreign materials (12). But their precise roles are not known, because of difficulty of isolation and a lack of suitable models.

Previously, we established a human monoblastic cell line, UG3, which has the following features: (1) GM-CSF- or IL-3-dependent proliferation, (2) GM-CSF- or M-CSF-induced differentiation to mature macrophages, (3) M-CSF- and IL-4-induced differentiation to MGCs with hydroxyapatite resorbing activity and TRAP activity (9). In this study, we showed that IL-13 as well as IL-4 had an ability to induce UG3 cells and PBMs to differentiate into MGCs, exclusively in the presence of M-CSF, whereas IL-2, IL-7 or IL-10 did not have such ability. Although IL-4- or IL-13-induced MGCs were characterized as FBMGCs but not osteoclasts, IL-4 or IL-13 induce markers of osteoclasts such as TRAP activity, vit-R expression and resorbing activity for hydroxyapatite in the absence of stroma cells. UG3 responded to IL-4 and IL-13 in the same way as normal human PBMs on MGC formation, and could be a suitable model for studying monocytic differentiation into MGCs and osteoclasts.

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FIG. 1. MGCs stained for TRAP. M-CSF-preincubated UG3 cells were cultured for two weeks in the presence of 100 ng/ml M-CSF and (a) 10 ng/ml IL-4 or (b) 10 ng/ml IL-13, then stained for TRAP. Magnification: 75-fold. Histochemical analysis was performed on cells obtained from four independent cultures.

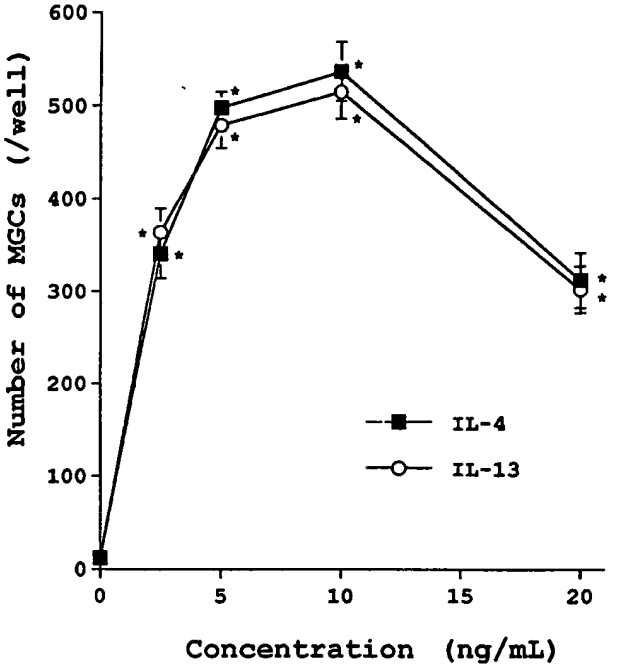

FIG. 2. Dose response effect of IL-4 and IL-13 on the generation of MGCs from UG3 cells. UG3 cells (3×10^5/ml) were cultured in 48-well culture plates with 100 ng/ml M-CSF in the presence or absence of various concentrations of IL-4 or IL-13. Error bars indicate standard deviation of cell numbers. *;$P<0.01$ compared with the number of MGC with M-CSF alone. Results are representative of three independent experiments.

MATERIALS AND METHODS

Cytokines. Recombinant human IL-3 was provided by Kirin Brewery Co., Ltd., Tokyo, Japan. Recombinant human M-CSF was provided by Morinaga Milk Industry Inc., Tokyo, Japan. Recombinant human GM-CSF, IL-2, IL-4 and IL-7 were purchased from Genzyme Corporation, Cambridge, MA. Recombinant human IL-10 and IL-13 were purchased from R&D Systems, Inc., Minneapolis, MN.

Cell preparation. PBMs were obtained from the peripheral blood of healthy volunteers after obtaining informed consent. Mononuclear cells were separated by Ficoll-Hypaque (Pharmacia LKB, Uppsala, Sweden, d=1.077) density gradient centrifugation at 400g for 30 minutes. The interphase containing mononuclear cells was harvested, then washed twice with phosphate-buffered saline (PBS) and once with fresh Iscove's modified Dulbecco's medium (IMDM) (GIBCO BRL, Gaithersburg, MD). The cells were cultured in IMDM supplemented with 5% fetal calf serum (FCS) (CSL Limited, Victoria, Australia) at 37°C in an atmosphere of 5% CO_2 in air for two hours. Adherent cells were harvested with trypsin-EDTA and washed twice with PBS and once with IMDM with 5% FCS as mature monocytes.

UG3 cells were maintained at a density of 2.5×10^5/ml in IMDM supplemented with 5% FCS and IL-3 (5 ng/ml) at 37°C in an atmosphere of 5% CO_2 in air. The cells were washed twice with PBS, then moved to IMDM containing 5% FCS and 100 ng/ml M-CSF. After a 10-day preincubation in the presence of M-CSF, the cells were harvested with trypsin-EDTA and washed twice with PBS and once with IMDM supplemented with 5% FCS. The PBMs or M-CSF-preincubated UG3 cells were cultured in new dishes in IMDM containing 5% FCS and 100 ng/ml M-CSF with or without IL-2 (100 U/ml), IL-4 (10 ng/ml), IL-7 (1 ng/ml), IL-10 (10 ng/ml) or IL-13 (10 ng/ml).

Assay for TRAP activity of the cells. After a 2-week culture with or without various cytokine combinations in 48-well culture plates, the cells were stained for TRAP with a commercially available kit (Sigma Diagnostics, St Louis, MO) according to the manufacturer's instructions. Cells containing at least 3 nuclei were considered MGCs.

Detection of PTH-R, cal-R and vit-R expression in MGCs. Reverse transcription-polymerase chain reaction (RT-PCR) was performed to detect PTH-R or cal-R messenger RNA (mRNA). Primers used were; PTH-Rs; 5'-AGGACGCTGTGCTCTACTCT-3', PTH-Ras; 5'-AGCTCTGACACTGACCCACA-3', cal-Rs; 5'-GCTTCTTTGTTGCGACCATC-3', cal-Ras; 5'-CTGGGAGGATGGAGAATACT-3'. Complementary DNA was synthesized by reverse transcription in a 80 μl reaction mixture containing 1 μg total cellular RNA, 150 μg/ml random hexanucleotide and 50 U reverse transcriptase (Seikagaku Co., Tokyo, Japan). A volume of 0.5 μl cDNA reaction mixture or control template was amplified in the presence of 1 U Thermus aquaticus DNA polymerase (Takara, Shiga, Japan), 25 mmol/l dNTP and 10 mmol/l of each specific primer in a total volume of 20 μl, on a Thermal Cycler (Perkin-Elmer Cetus, Norwalk, CT). One PCR cycle consisted of denaturation at 95°C for 1 minute, primer annealing at 62°C for 1 minute, and extension at 72°C for 2 minutes, and this cycle was repeated 40 times. An aliquot of 6 μl of each PCR product was electrophoresed in 3% agarose (NuSieve, FMC BioProducts, Rockland, ME), and stained with ethidium bromide. For detection of vit-R, immunostaining was performed using monoclonal antibody 23c6 (ENDOGEN, Woburn, MA) and Histofine detection system (Nichirei Co. Ltd., Tokyo, Japan) according to the manufacture's instructions.

Analysis of hydroxyapatite-resorption activity. The cells which were prepared as described in "Cell preparation" were seeded on plastic slides covered with hydroxyapatite (Osteologic, Millenium

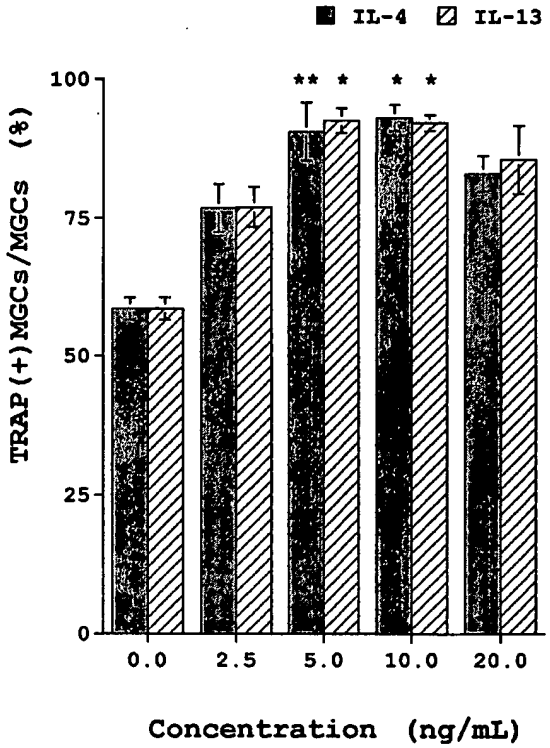

FIG. 3. Dose response effect of IL-4 and IL-13 on the percentage of TRAP positive MGCs in all MGCs. UG3 cells (3×10^5/ml) were cultured in 48-well culture plates with 100 ng/ml M-CSF in the presence or absence of various concentrations of IL-4 or IL-13. Error bars indicate standard deviation of percentage. *;$P<0.01$, **;$P<0.05$ compared with the percentage in the presence of M-CSF alone. Results are representative of three independent experiments.

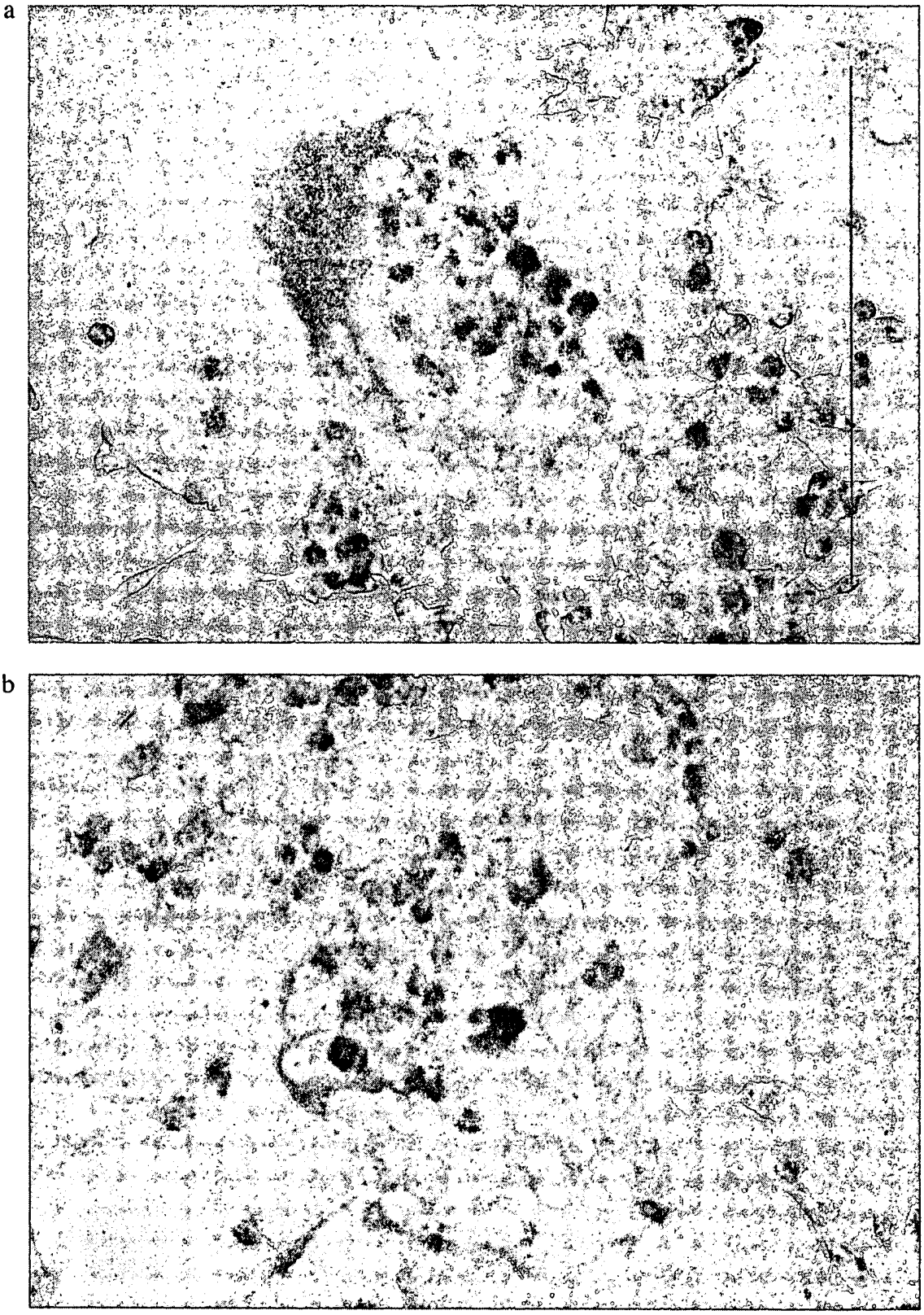

FIG. 4. Vit-R expression on the cells. Vit-R expression was determined by immunohistochemistry on UG3 cells cultured with (a); 100 ng/ml M-CSF and 10 ng/ml IL-4, (b); 100 ng/ml M-CSF and 10 ng/ml IL-13 or (c); 100 ng/ml M-CSF alone for two weeks. Magnification: 200-fold. Histochemical analysis was performed with samples taken from three independent cultures.

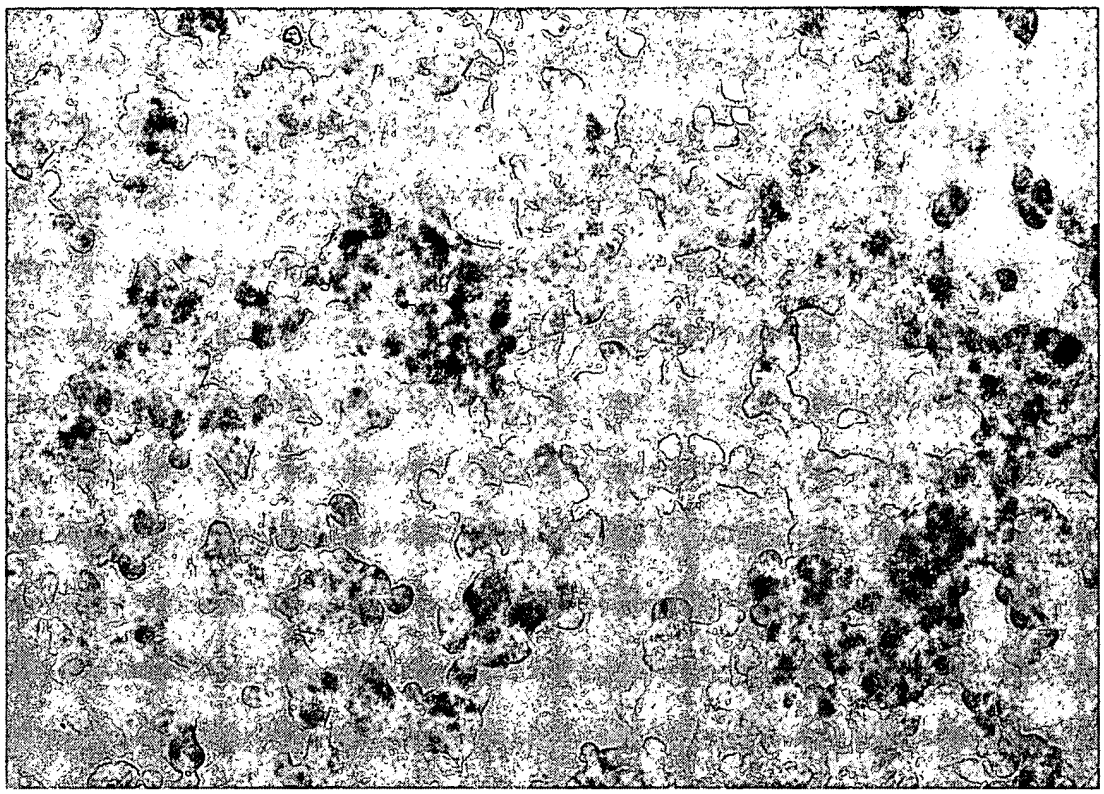

FIG. 4—*Continued*

Biologix Inc., Ontario, Canada). After a 2-week culture, the cells were removed and subjected to von Kossa staining (15).

Statistical analyses. Statistical analyses were performed with paired t-tests.

RESULTS

Morphology and TRAP activity of IL-4- or IL-13-induced MGCs in the presence of M-CSF. UG3 cells, preincubated in the presence of 100 ng/ml M-CSF for 10 days, formed MGCs with TRAP activity after a 2-week culture in IMDM with 5% FCS supplemented with combinations of M-CSF (100 ng/ml), and IL-4 (10 ng/ml) or IL-13 (10 ng/ml) (Fig. 1a or 1b, respectively). Both the MGCs induced by IL-4 and IL-13 in the presence of M-CSF were similar in morphology. PBMs also formed MGCs with TRAP activity after a 2-week culture with IL-4 or IL-13 in the presence of M-CSF (data not shown). The morphology of MGCs derived from UG3 cells and PBMs was the same. PBMs and UG3 cells did not form MGCs after incubation with IL-2 (100 U/ml), IL-7 (1 ng/ml) or IL-10 (10 ng/ml) in the presence of M-CSF (100 ng/ml) (data not shown). IL-4 (10 ng/ml) or IL-13 (10 ng/ml) induced UG3 cells and PBMs to aggregate but not to form MGCs without M-CSF, even when IL-3 (5 ng/ml) or GM-CSF (1 ng/ml) were added in the culture medium instead of M-CSF (data not shown).

Dose-dependent effects of IL-4 and IL-13. MGC formation from UG3 was maximum when the concentration of IL-4 or IL-13 was 10 ng/ml (Fig. 2). The percentage of TRAP-positive MGCs reached plateau at 5 ng/ml of IL-4 or IL-13 (Fig. 3). Based on these results, all the following experiments were conducted at the concentration of 10 ng/ml of IL-4 or IL-13.

Expression of cal-R, PTH-R and vit-R. MGCs differentiated from UG3 cells stained positive for vit-R when cultured with combinations of M-CSF (100 ng/ml) and either of IL-4 (10 ng/ml) (Fig. 4a) or IL-13 (10 ng/ml) (Fig. 4b). On the other hand, UG3 cells cultured in the presence of M-CSF (100 ng/ml) alone were negative for vit-R (Fig. 4c). Normal PBMs were also positive for vit-R when cultured with M-CSF and either of IL-4 or IL-13, but were negative when cultured with M-CSF alone (data not shown). No messenger RNA for cal-R or PTH-R was detected by RT-PCR in MGCs cultured with any of the combinations of cytokines from UG3 cells or PBMs (data not shown).

Hydroxyapatite resorption activity of MGCs. In the presence of M-CSF (100 ng/ml) and IL-4 (10 ng/ml) (Fig. 5a) or M-CSF (100 ng/ml) and IL-13 (10 ng/ml) (Fig. 5b), some of the MGCs differentiated from UG3 cells cultured in Osteologic slide flasks resorbed hydroxyapatite, while none of those cultured with M-CSF (100 ng/ml) alone did (Fig. 5c). UG3 cells cultured with IL-4 or IL-13 did not resorb hydroxyapatite in the presence of IL-3 or GM-CSF instead of M-CSF (data

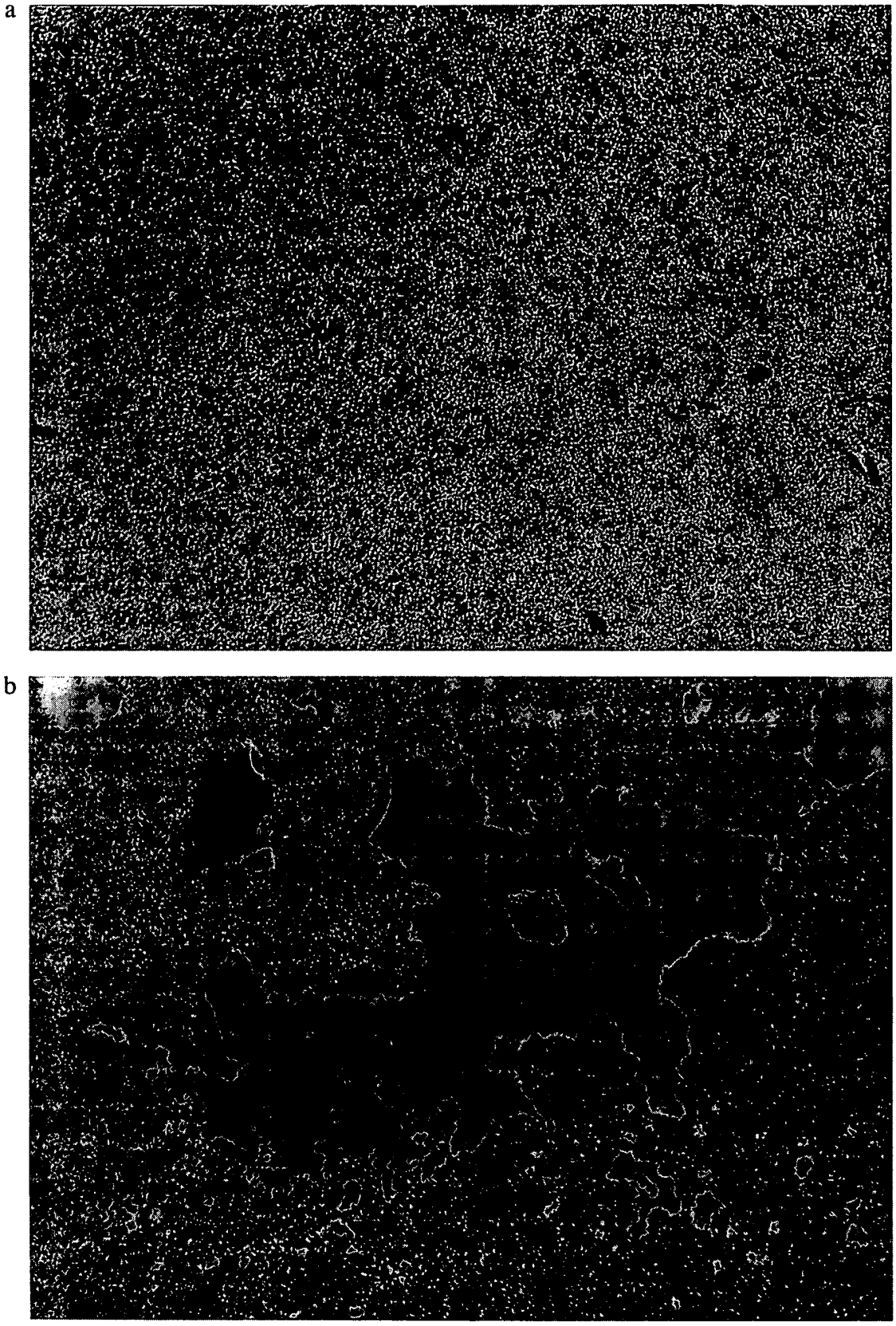

FIG. 5. Resorption of hydroxyapatite by MGCs exposed to M-CSF and IL-4 or IL-13. Hydroxyapatite was stained black by von Kossa staining. UG3 cells had pericellular clear areas resulting from hydroxyapatite resorption when cultured in the presence of (a) 100 ng/ml M-CSF and 10 ng/ml IL-4 or (b) 100 ng/ml M-CSF and 10 ng/ml IL-13, while those cultured in the presence of (c) 100 ng/ml M-CSF alone did not. Magnification: 50-fold. Analysis was performed with cells obtained from three independent experiments.

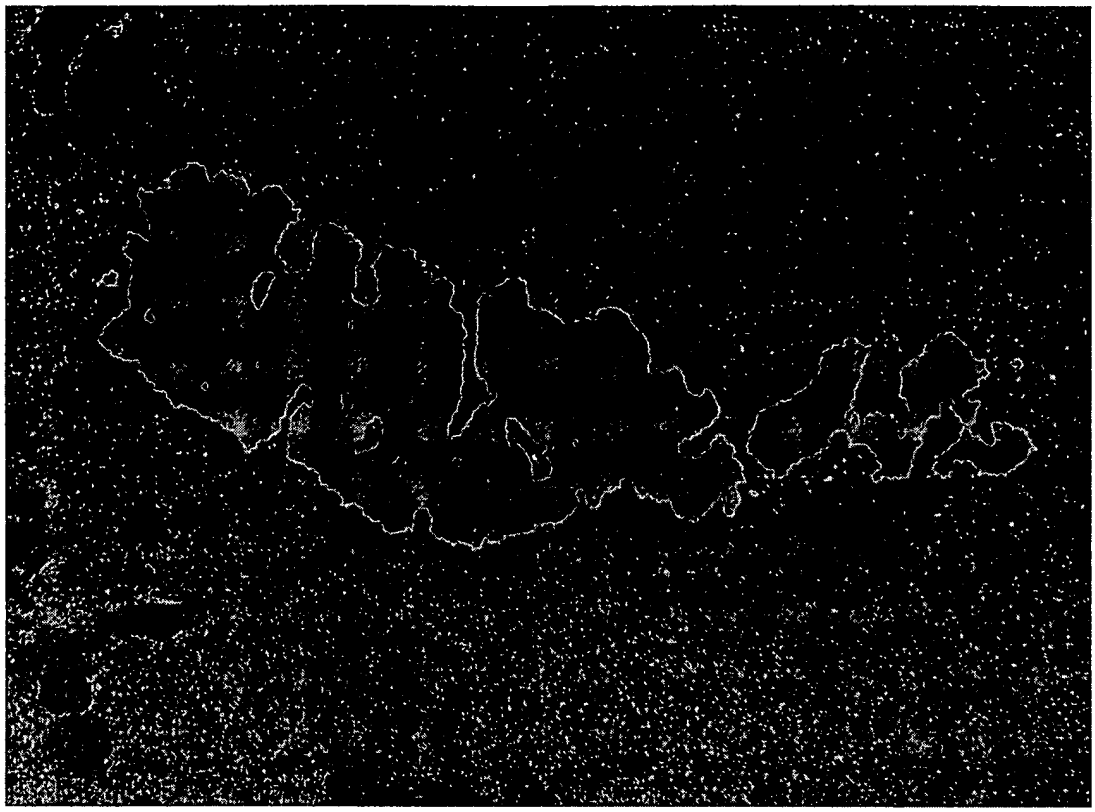

FIG. 5—*Continued*

not shown). Normal PBMs behaved in the same way as UG3 (data not shown).

DISCUSSION

In the present study, we showed that both IL-4 and IL-13 induced UG3 cells as well as PBMs to differentiate into MGCs, exclusively in the presence of M-CSF. With regard to MGC formation, some investigators reported that IL-4 alone was enough to induce formation of MGCs from rat microglia (16) and human monocytes/macrophages (17), but others reported that other growth factors were needed for MGC formation from human monocytes/macrophages (7-9). Akagawa reported that a combination of GM-CSF and IL-4 did not induce MGC formation (5), but McNally and McInnes reported that IL-3 and GM-CSF stimulate IL-4-induced MGC formation (7, 17). Recently, DeFife reported that IL-13 also induced human monocytes/macrophages to form MGCs in the absence of other growth factors (6). In the present study, both IL-4 and IL-13 induced UG3 cells and PBMs to differentiate into MGCs in the presence of M-CSF, while they did not in the presence of GM-CSF or IL-3 instead of M-CSF. Our results suggest that the presence of M-CSF is essential to IL-4- and IL-13-induced MGC formation from monocytes/macrophages. The discrepancy in the results may reflect different cell source or culture conditions.

Previous studies demonstrated that IL-2, IL-4, IL-7, IL-9, IL-13 and IL-15 share the common gamma chain of IL-2 receptor (2, 18). Interestingly, IL-2, IL-7 and IL-10 did not show activities to form MGCs from either UG3 cells or PBMs, whereas IL-4 and IL-13 did. It can be postulated that the signal transduction pathway of IL-2/IL-7 in MGC formation from monocytes/macrophages is different from that of IL-4/IL-13 as recently reported for lymphoid cells (19) and for osteoblast-like cells (20).

Intercellular adhesion molecule-1 (ICAM-1)/leukocyte function-associated antigen-1 (LFA-1) (21), macrophage mannose receptor (MMR) (23) and class II major histocompatibility complex (MHC) (24) are suspected to be essential in IL-4-induced MGC formation from monocytes/macrophages. DeFife reported that IL-13 acts independently of IL-4 on MGC formation (6). Although the alpha chain of IL-4 receptor is a component of IL-13 receptor (24), the presence of another possible IL-13 receptor component was also suggested (25). The details of IL-4- or IL-13-induced MGC formation have yet to be elucidated, and suitable models are needed to further investigate the mechanism of IL-4- or IL-13-induced MGC formation.

Expression of cal-R and/or PTH-R and bone-resorbing activity are essential characteristics to define osteoclasts (13). Because of the lack of mRNA of

cal-R or PTH-R, our MGCs induced by IL-4 or IL-13 in the presence of M-CSF could not be considered as osteoclasts. Takahashi reported that direct contact with osteoblastic cells is essential for monocytes to differentiate into osteoclasts (26), and other reports showed that IL-4 and IL-13 inhibited the formation of TRAP-positive MGCs in the culture of whole bone marrow cells or coculture of bone marrow cells with osteoblastic cells (20, 27). In our study, IL-4- and IL-13-induced MGCs displayed TRAP activity, vit-R expression and hydroxyapatite resorbing activity without coculturing with stroma cells. Furthermore, IL-4 and IL-13 enhanced TRAP-positivity of these MGCs (Fig. 2, 3). Although these IL-4- and IL-13-induced MGCs in the presence of M-CSF lack cal-R and PTH-R, and therefore are distinct from osteoclasts, these MGCs might have already been committed to osteoclasts as reported by Pandey et al (28) and Quinn et al (29). These results suggest possible initiation by IL-4 and IL-13 in osteoclasts development from monocytes/macrophages.

UG3 is the first human cell line which forms MGCs induced by IL-4 and IL-13 in the same way as normal human PBMs, and is a useful tool with which to further investigate the function and signal transduction of IL-4 and IL-13 in monocytes/macrophages including mechanisms of formation of MGCs, physiology of MGCs and the relationship between these MGCs and osteoclasts.

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